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Hydrolytically Stable Titanium-45

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Introduction

Titanium-45, a candidate PET isotope, is underemployed largely because of the challenging aqueous chemistry of Ti(IV). The propensity for hydrolysis of Ti(IV) compounds makes radiolabeling difficult and currently excludes ^{45}Ti from use in bio-conjugate chemistry. This is unfortunate because the physical characteristics are extremely desirable (FIG. 1): ^{45}Ti has a 3 hour half-life, a positron branching ratio of 85%, a low E_{max} of 1.04 MeV, and negligible secondary gamma emission[1]. In terms of isotope production, ^{45}Ti is transmuted from naturally monoisotopic ^{45}Sc by low energy proton irradiation. The high cross-section (300-400 mb from 7.8-14.4 MeV[2]) and resulting production rates on an unenriched metal foil target contribute to make ^{45}Ti an ideal PET radionuclide.

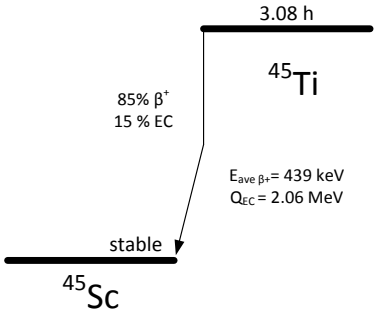

FIGURE 1: The simplified decay scheme of ^{45}Ti

In order to even bring ^{45}Ti to a preclinical setting, the hydrolytic instability of aqueous Ti(IV) needs to be addressed.

Titanium citrate is a good example of a Ti(IV) complex that is hydrolytically stable at physiological pH[3], and is the basis for two of the very few preclinical radio-tracing publications employing ^{45}Ti[4,5]. The results from Vavere and Welch[4] showed that ^{45}Ti administered as citrate rapidly transchelated to transferrin (Tf) in serum. While this property proved useful for tracking the distribution of Tf, it precludes the use of Ti-citrate as a platform for bioconjugate chemistry.

Recently, the groups of Edit Tshuva (Hebrew University of Jerusalem) and Thomas Huhn (University of Konstanz) have synthesized several hydrolytically stable Ti(IV) compounds based upon the salan ligand (a diamine bisphenolato complex)[6–9]. Interestingly, many of these compounds have shown heightened cytotoxicity against HT-29 and other cell lines compared to traditional metal-based chemotherapeutics such as cis-platin[8].

The salan-based titanium compounds prompt two interesting topics for radiochemical investigation. First, the biodistribution of these antiproliferative agents may be traced with ^{45}Ti. Second, the ligand system may be exploited as a scaffold for bioconjugate chemistry with ^{45}Ti.

The aim of our work is to investigate different methods of producing the radioactive analogue of one of these Ti(IV)-salan compounds, Ti-salan-dipic[7] (depicted in FIG. 2), which has hydrolytic stability on the order of weeks, and is TLC-traceable. In the current report we present some of the methods we surveyed to separate ^{45}Ti from irradiated scandium. Reactions with salan and dipic (pyridine-2,6-dicarboxylic acid) are presented as assessments of the chemical viability of the extracted ^{45}Ti by each method.

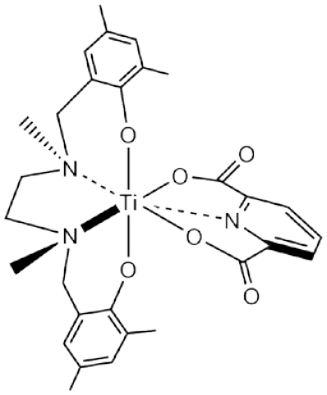

FIGURE 2: A structural depiction of Ti-salan-dipic

Material and Methods

^{45}Ti was produced by proton irradiation of 250 µm scandium foils (Alfa Aesar) at currents ranging from 10-20 µA on a GE PETTrace. A 500 µm aluminum degrader was used to take the proton energy down from the nominal 16.5 MeV. The scandium was cooled by contact to a water-cooled silver plate.

The activated foil was dissolved in 4 M HCl, dried under argon at 120 °C, and taken back up in 12 M HCl. The intention of using 12 M HCl was to restrict hydrolysis in the aqueous environment, and revert any titanyl ions to Ti(IV) chlorides [10]. Next, four (*i-iv* below) different approaches to

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recovering the ^{45}Ti from the aqueous environment were taken with varying success. Radiochemical purity and reaction yield was assessed with silica TLC in 1:1 chloroform : ethyl acetate. When necessary, a co-spot of non-radioactive Ti-salan-dipic (clearly visible, distinctive orange color) was added in order to confirm the R_f value, which varied from 0.53-0.60 depending on residual solvents.

i. Hydroxamate Resin:

^{45}Ti was separated on hydroxamate resin, as presented by K. Gagnon[11] with minor modifications. Briefly, hydroxamate resin was prepared as described by Holland *et al.*[12], and washed with 12 M HCl. The dissolved and reconstituted Sc (16 mg) plus ^{45}Ti in 12 M HCl (1 mL) was passed slowly over approximately 100 mg resin in an 8mm ID column with polyethylene frits. Flow was controlled by gravity. Salan in pyridine (20 mg, 1 mL) was passed through, followed by addition of dipic in pyridine (10 mg, 1 mL) while heating. The lability of ^{45}Ti remaining on the column was determined by passing 1 M citric acid over the column and trapping the eluted activity on a QMA Sep-Pak cartridge (Waters).

ii. Solvent Extraction:

^{45}Ti was extracted into 1-octanol following the method reported by Siikanen *et al*[13]. First, the activity and Sc laden HCl (2.8 mg in 1.1 mL) was vortexed with 1-octanol (3 mL) and allowed to settle. To strip the Sc, the aqueous phase was removed, and replaced with 1 mL of fresh 12 M HCl. The mixture was vortexed again, settled, and the aqueous phase removed. Then the organic phase was stripped again and settled by centrifugation. Finally, 1 mL of the organic phase was used directly for radiolabeling by addition of 1 mL salan in pyridine (50 mg/mL) followed by mixing with 1 mL dipic in pyridine (23 mg/mL) at 60 ^{o}C for 15 minutes.

iii. Hydrophobicity Pairing:

As a next approach, the solvent extraction method was transferred to solid phase. Here, ^{45}Ti was trapped on a C-18 cartridge that had been pre-loaded with 1-hexanol, similar to ion-pairing. The C-18 was prepped with 1 mL 1-hexanol, and then washed with 0.5 mL 12 M HCl. The Sc plus ^{45}Ti in HCl (7 mg Sc, 200 µL) was passed over the cartridge, followed by 1 mL HCl wash. The cartridge was eluted with 1 mL isopropanol. Salan in pyridine (63 mg/mL) and dipic in pyridine (32 mg/mL) were added to the isopropanol, and mixed for 15 min at 60 ^{o}C.

iv. Extraction onto solid supported 1,3 diol:

158 mg Hypogel 200 diol (RAPP polymers) was equilibrated for 20 minutes in 12 M HCl, and packed into an 8 mm ID column with polyethylene frits. 500 µL of reconstituted Sc/^{45}Ti was added to the top, and dripped through with light pressure (approximately 1 drop per minute). The resin was then washed with 500 µL more HCl and rinsed with dry acetonitrile.

Next, 1 mL salan in pyridine (60 mg/mL) was pushed through slowly, followed by dipic in pyridine (1 mL, 30 mg).

Results and Discussion

The results and observations from each method described above are given in the next sections:

i. Hydroxamate Resin:

Upon addition of the dissolved Sc to the column, the top of the resin showed a bright yellow band, and the activity was almost quantitatively trapped (99%). Addition of salan and dipic had no apparent effect upon the ^{45}Ti, which was not moved from the resin in either case. Washes with pyridine and methanol also did not remove activity from the column as would be expected if the [^{45}Ti] Ti-salan-dipic had been successfully synthesized.

As a test, the resin was washed with 1M citric acid with the eluent passing over a QMA Sep-Pak. In our experience citric acid removes large quantities of unreacted ^{45}Ti from the resin in a form that can be trapped on the QMA resin. This characteristic behavior was observed in the current case, indicating that the hydroxamate resin restricted reaction with salan and dipic, but did not irreversibly bind the titanium.

ii. Solvent extraction:

The solvent extraction method did not deviate from the expected behavior. Overall, after three emulsions and settlings, the organic phase contained 48% of the ^{45}Ti activity. Because the organic phase was used directly for synthesis, back extraction methods were not tested.

Upon addition of salan in pyridine, white fuming was observed. This is likely due to the quenching of residual HCl in the organic phase by the basic pyridine. The reaction yield was 53%, giving an overall yield of 25%. The incomplete reaction can possibly be due to hydrolysis of Ti from residual water in the octanol after the extraction process. Additionally, 1-octanol presented a problem on the TLC by splitting the product peak. This splitting was also observed with the non-radioactive, non-trace, compound in the presence of octanol, and was therefore attributed to an octanol artifact.

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iii. Hydrophobicity pairing:
After some success with the solvent extraction method, a new approach was taken based on the same chemistry. The aim was to perform the solvent extraction on solid phase, thereby limiting radiation exposure during phase mixing, and restricting the volume of long-chain alcohol that would be present in the labeling solution.

In this method, 1-hexanol was passed over a C-18 plus Sep-Pak in order to functionalize it. The concept was similar to ion-pairing used in HPLC chromatography, where the functionality of a reversed-phase column is altered by hydrophobic interactions with a molecule bearing a lipophilic side-chain and the analyte-interacting moiety. When the activity and Sc in HCl was passed over the resin, 33% of the titanium was trapped (a control experiment without 1-hexanol gave negligible extraction of ^{45}Ti). This was easily eluted with 100% efficiency by interrupting the hydrophobic interaction between 1-hexanol and the solid phase with 1mL of isopropanol. Reaction with the ligands converted 86% of the ^{45}Ti to [^{45}Ti]Ti-salan-dipic.

iv. Extraction onto solid supported 1,3 diol:
The 1,3-diol resin did not swell, nor did it appear to wet very efficiently in HCl. Also, passage of the Sc/HCl solution was slow under gravity, and had to be aided with occasional light pressure. Nevertheless, the resin trapped 96% of the ^{45}Ti that was applied to it. Pyridine caused significant swelling of the resin.

The reaction with salan removed 15% of the activity, and caused the solid phase to turn a bright yellow color. The eluent was a deep blue-violet color, which was later found to be due to Fe (by ICPOES measurement), presumably as a soluble salan complex. The reaction with dipic caused release of 40% of the ^{45}Ti, with >70% being present as the final product [^{45}Ti]Ti-salan-dipic. In follow-up experiments using this method, we found that heating the recovered eluent leads to full conversion.

Summary

The trap, release, and yields for the four methods listed above are shown in TAB. 1. Although the overall yields are very similar for methods *ii*, *iii*, and *iv*, the most promising result was with the 1,3-diol resin because it had the added advantage of reacting on-column, and releasing on addition of the dipic ligand. This is an important point for moving forward with a pure production of [^{45}Ti]Ti-salan-dipic, because it allows removal of excess salan ligand –a challenging purification due to the similarities between Ti-salan-dipic and salan itself. The other methods appear to be reasonable approaches for isolating ^{45}Ti, but in the specific situation of reactions with salan and dipic, they are not the best choice.

	Method	%		
		Trap	Release	Yield
i	SPE HX.	99	0	0
ii	Solv.extr.	48	-	25*
iii	SPE / hex.	33	100	26
iv	SPE diol	96	40	28

TABLE 1. Results for trap and release of ^{45}Ti and subsequent labeling yield (as percentage of activity before trapping, not decay corrected) of [^{45}Ti]Ti-salan-dipic for conditions described above. *The presence of 1-octanol caused abnormal chromatography (peak splitting).

Conclusion

We conclude that the hydrolytically stable ^{45}Ti compound [^{45}Ti]Ti-salan-dipic can be synthesized in high yield after trapping of ^{45}Ti on a 1,3-diol resin, and reacting on-column. Additionally, it is possible that the other methods presented for isolating ^{45}Ti out of HCl mixtures may find use in other applications requiring different solvents or employing different ligands. We hope that this work adds to the base-knowledge of ^{45}Ti radiochemistry, and helps push ^{45}Ti toward more widespread applications.

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